AD_____

Award Number: W81XWH-11-1-0427

TITLE: Improving Outcome in Malignant Pleural Mesothelioma (MPM) Using Pulsed-Protracted External Beam Radiation (PERT) and Intrapleural Delivery of Stem Cells

PRINCIPAL INVESTIGATOR: Brian Marples, PhD

CONTRACTING ORGANIZATION: William Beaumont Hospital Inc
Royal Oak, MI 48073

REPORT DATE: July 2013

TYPE OF REPORT: Annual Report

PREPARED FOR: U.S. Army Medical Research and Materiel Command
Fort Detrick, Maryland 21702-5012

REPORT DOCUMENTATION PAGE

Form Approved
OMB No. 0704-0188

1. REPORT DATE	2. REPORT TYPE	3. DATES COVERED
July 2013	Annual Report	1 July 2012 – 30 June 2013

4. TITLE AND SUBTITLE

Improving Outcome in Malignant Pleural Mesothelioma (MPM) Using Pulsed-Protracted External Beam Radiation (PERT) and Intrapleural Delivery of Stem Cells

5a. CONTRACT NUMBER

5b. GRANT NUMBER
W81XWH-11-1-0427

5c. PROGRAM ELEMENT NUMBER

6. AUTHOR(S)

Brian Marples, PhD

E-Mail: brian.marples@beaumont.edu

5d. PROJECT NUMBER

5e. TASK NUMBER

5f. WORK UNIT NUMBER

7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES)

William Beaumont Hospital Inc
3601 W 13 Mile Road
Royal Oak, MI 48073

8. PERFORMING ORGANIZATION REPORT NUMBER

9. SPONSORING / MONITORING AGENCY NAME(S) AND ADDRESS(ES)
U.S. Army Medical Research and Materiel Command
Fort Detrick, Maryland 21702-5012

10. SPONSOR/MONITOR'S ACRONYM(S)

11. SPONSOR/MONITOR'S REPORT NUMBER(S)

12. DISTRIBUTION / AVAILABILITY STATEMENT
Approved for Public Release; Distribution Unlimited

13. SUPPLEMENTARY NOTES

14. ABSTRACT
Malignant Pleural Mesothelioma (MPM) survival remains poor despite multidisciplinary treatment involving aggressive surgery, chemotherapy and adjuvant radiotherapy (RT). The large RT treatment volume, and concerns about the proximity of radiosensitive normal structures, restricts the tumoricidal dose of radiotherapy that can be delivered. These concerns limit the effectiveness of adjuvant RT. To overcome this limitation, an entirely novel radiation treatment schedule in combination with post-RT delivery of bone marrow-derived stem cells was examined to improve tumor control and facilitate normal tissue proliferation. A rat model of MPM was used. The RT regime consisted of 10 pulses of low-dose RT (0.2 Gy) using a 3 minute inter-pulse interval (PERT) to a daily dose of 2 Gy. The inclusion of post-RT stem cell therapy is to repopulate normal tissues in the RT field. RT tumor response was assessed by microPET/CT (Positron emission tomography/computed tomography) imaging. In vitro cell survival data was used to demonstrate PERT was not inferior to standard RT (2 Gy single continuous treatments). In vivo, The surgical procedure has been established and tumor model has been established and tumor volume determined by in situ with F18-FDG. Unexpected technological problems with respect to the microPET scanner have slowed the imaging aspect of the project. However, to date, we have demonstrated that RT is effective at reducing MPM tumor growth in vivo; and this is associated with recruitment of hematological stem cells. Studies are currently on-going to determine if PERT is superior to standard RT in MPM.

15. SUBJECT TERMS
Malignant Pleural Mesothelioma, Low dose pulsed radiotherapy, stem cells, animal imaging.

16. SECURITY CLASSIFICATION OF:			17. LIMITATION OF ABSTRACT	18. NUMBER OF PAGES	19a. NAME OF RESPONSIBLE PERSON
a. REPORT	b. ABSTRACT	c. THIS PAGE			USAMRMC
U	U	U	UU	7	19b. TELEPHONE NUMBER *(include area code)*

Table of Contents

Page

ABSTRACT

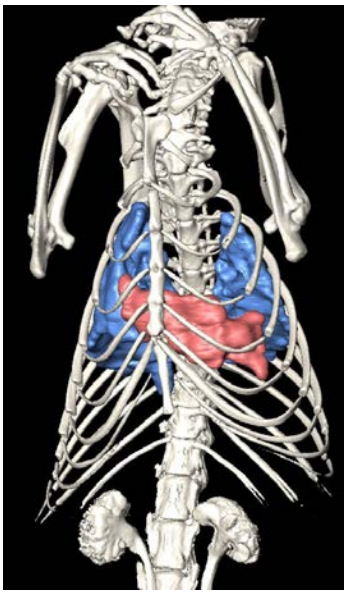
Malignant Pleural Mesothelioma (MPM) survival remains poor. Aggressive surgery, chemotherapy and adjuvant radiotherapy (RT) are often ineffective. The efficacy of the RT component within the multimodality therapy is confounded by the large RT treatment volume. Consequently, radiosensitive normal structures proximal to the tumor are irradiated which restricts the size of the tumoricidal dose of radiotherapy that can be delivered to the tumoe. To improve RT effectiveness, we investigated an entirely novel radiation treatment schedule in combination with post-RT delivery of bone marrow-derived stem cells. A rat model of MPM was used. The RT regime consisted of 10 pulses of low-dose RT (0.2 Gy) using a 3 minute inter-pulse interval (PERT) to a daily dose of 2 Gy. The inclusion of post-RT stem cell therapy functions to repopulate normal tissues in the RT field. RT tumor response was assessed by microPET/CT (Positron emission tomography/computed tomography) imaging. In vitro cell survival data demonstrated PERT was not inferior to standard RT (2 Gy single continuous treatments). In vivo, the surgical procedure and tumor model were established and tumor volume determined by *in situ* with F18-FDG; although many of the tumors then regressed in the absence of complete RT treatment. Tumor take rate in the original rat strain was low and those tumors that did develop naturally regressed. Moreover, the original tumor placement was difficult to distinguish via PET imaging as the tumors were located adjacent to the heart which is a highly PET-avid organ. Therefore, after minor alterations to the surgical implant procedure in the original rat strain under the guidance of the attending veterinarian failed to alleviate these problems, a decision was made to change to a more immune compromised rat strain (described below). The revised rat strain improved the stability and reliability of the in vivo model; both orthotopic and subcutaneous implanted tumors. In vivo studies are currently on-going to determine if PERT is superior to standard RT in MPM.

INTRODUCTION

Malignant Pleural Mesothelioma (MPM) survival remains poor despite multidisciplinary treatment involving aggressive surgery, chemotherapy and adjuvant radiotherapy (RT). The large RT treatment volume, and concerns about the proximity of radiosensitive normal structures, restricts the tumoricidal dose of radiotherapy that can be delivered. These concerns limit the effectiveness of adjuvant RT. We propose to deliver the radiotherapy using an entirely novel treatment schedule and combine this with post-RT local-delivery of bone marrow-derived stem cells to facilitate normal tissue proliferation. The concept is to deliver 10 pulses of low-dose RT (0.2 Gy) using a 3 minute inter-pulse interval (PERT) to introduce the RT-induced damage at a level that evades ATM dose-dependent DNA damage detection and repair mechanisms. Post-RT stem cell therapy is to repopulate normal tissues in the RT field.

BODY

The surgical procedure has been established in the new rat strain (Fig. 1). Animals are anesthetized, surgically-implanted with tumor cells and recovered. These tumors were imaged *in situ* with F18-FDG (Fig. 1). The tumors were evident in the pleural cavity (Fig. 2). We are now



Representative orthotopic mesothelioma tumor. The lungs are pseudo-colored blue and red color indicates the mesothelioma tumor.

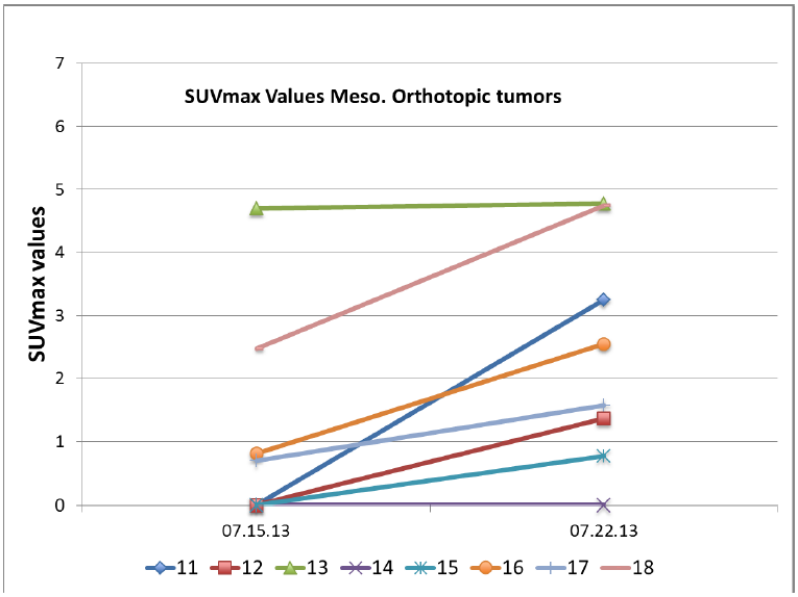


Figure 2: Change in growth rate as indicated by SUVmax for mesothelioma tumors implanted in animals #11-#18. Tumor volume increased over the time frame 07.15.13 to 07.22.13

able to reproducibility establish the model (Fig. 3).

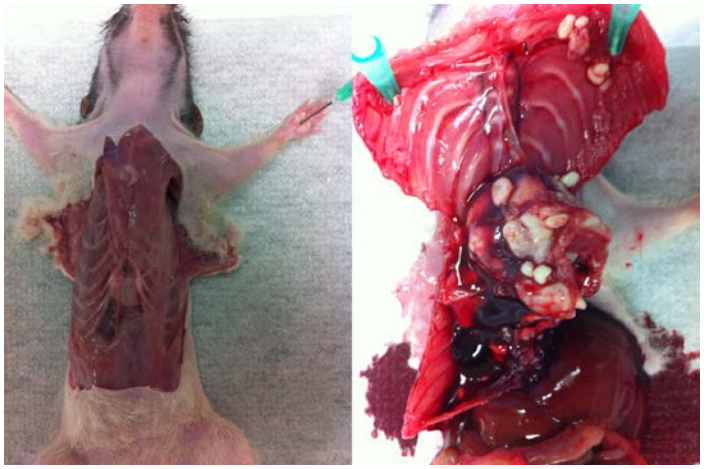


Figure 3: Mesothelioma tumors in the plural cavity and invading the lung.

Our experiments have progressed with some success recently; although the earlier experiments did not go as we had hoped. We still have animals currently on study with ten orthotopically-implanted mesothelioma tumors awaiting treatment (Fig. 3); and additional experiments planned. This orthotopic tumor model has taken much longer to establish and grow successfully than we anticipated, months rather than weeks and has involved in a change in host strain. This project was delayed by two major issues.

The replacement of John Chunta PhD with Sarah Krueger PhD. Dr. Krueger was recently re-trained to operate the microPET scanner and perform the imaging assays after Dr. Chunta left unexpectedly for another position.

Then, Dr. Krueger established that a change in surgical procedure was needed to a less invasive implantation technique. As explained in more detail below, the routine tissue recovery and repair that occurs in the animal following the original surgical procedure was evident on the microPET images, which confounded tumor identification. This problem was resolved by markedly reducing the invasiveness of the implant procedure under the guidance of the attending veterinarian. Each change to the surgical procedure was achieved using one animal at a time. Consequently, small iterations in the surgical procedure took months to establish since the growth rate of the tumor was slow, and further iterations could not occur until the previous animal had been allowed sufficient time for the tumor to grow and develop, or not. The veterinarian only allowed individual animals to be implanted until the technique was successfully established, as a complex survival-surgery procedure was required; this limited the rate of progress.

We have evaluated the radiation procedures of administering a low-dose pulsed treatment regime using the micro-irradiator. Animals are anesthetized during irradiation procedure (10 doses of 0.2 Gy given daily for 5 consecutive days) with 2-3% isoflurane; this procedure has been defined and is well-tolerated. This procedure is now established.

The SARRP treatment plan has been developed and used to treat orthotpic lung animals with standard 2 Gy treatments and PERT treatments (Fig. 4).

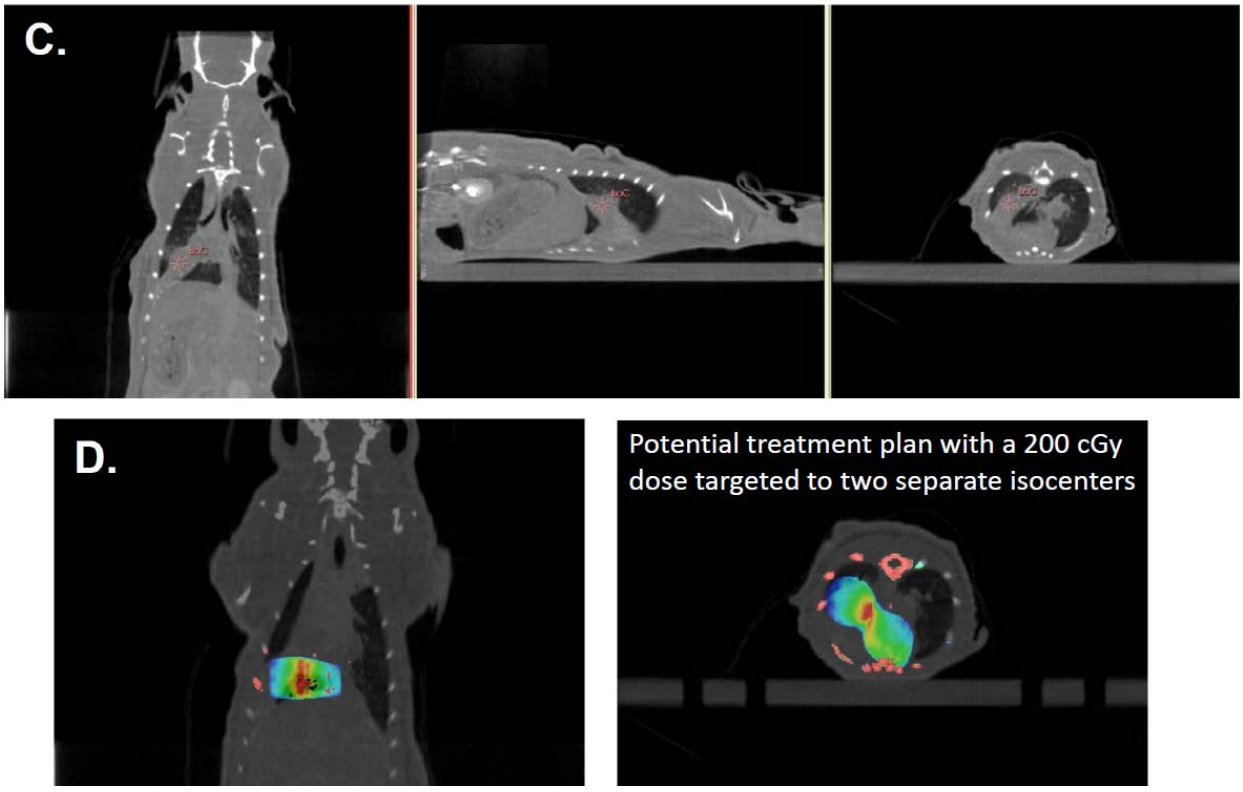


Figure 4. CT image indicating orthotopic mesothelioma tumors (panel C red star) and radiation treatment plan indicating pseudocolored radiation dose. Red color indicates 2 Gy to the tumor volume.

KEY RESEARCH ACCOMPLISHMENTS

- Measure survival response of MPM cells in vitro
- Demonstrated PERT is not inferior to standard RT in vitro
- Established animal model with a change in rat strain to improve tumor reliability
- Demonstrated RT reduces MPM growth in vivo and growth rate in a consistent manner
- Planned treatments on the SARRP.

REPORTABLE OUTCOMES

At present, the work is on-going and a no-cost extension has been requested. Therefore the work has not been published or presented, but a methodology paper is in preparation. The tumor imaging and radiation treatment delivery was presented as a component of the research paper at the World Molecular Imaging conference by Dr. Sarah Krueger.

CONCLUSIONS

The experiments are still on-going. We have demonstrated that PERT is not inferior to standard RT (the current standard of care for MPM). The preliminary in vivo data are supportive of this conclusion. The necessary change in rat strain has improved the functionality of the mdoel.

FUTURE EXPERIMENTS

Animals with orthotopic tumors will be irradiated using two RT schedule (single 2 Gy fraction per day) or PERT (10 x 0.2 Gy to same daily dose) with or without stem cell delivery (n=10 per arm) to a total dose of 30 Gy. Treatment response will be evaluated and harvested tissues examined for incorporated stem cells using the techniques described above.

TIME LINE

We expected the remainder of the experiments to be complete within the next few months as all the assays and procedures are now established.

REFERENCES AND APPENDICES

None. Studies are on-going and data is still being compiled for publication.

WITH RESPECT TO STATEMENT OF WORK

Specific Aim #1 – Establish and treat Mesothelioma model (Months 1-7)
Overview. The aim is to develop Mesothelioma model and treat with pulsed radiotherapy.

Subtask1: Establish surgical technique and tumor implantation (Months 1-2)

a. Purchase and acclimatize 12-week old rats.
b. Establish surgery procedure for intrapulmonary implanting of Mesothelioma cells.
c. Ensure successful infection-free surgery without adverse pulmonary breathing rates or events.

The work from Subtask1 is complete.

Subtask2: Compare Radiation schedules (Months 2-7)

a. Measure breathing rate and lung function in tumor-bearing animals
b. Obtain weekly blood samples
c. Treat with conventional fractionated radiotherapy and pulse schedules
d. MicroPET scan animals to assess treatment outcomes
e. Harvest lungs and other tissue for histopathology
f. Analyze data set to determine effectiveness of two RT schedules.

The work from Subtask2 is complete for untreated animals. Task f have not been completed but is underway.

Subtask3: Compare Radiation schedules (Months 2-10)

g. Measure breathing rate and lung function in tumor bearing animals
h. Obtain weekly blood samples
i. Treat with conventional fractionated radiotherapy and pulse schedules ***plus stem cell therapy***
j. MicroPET scan animals to assess treatment outcomes
k. Harvest lungs and other tissue for histopathology
l. Analyze data set to determine effectiveness of two RT schedules.

Tasks g, h, k, have been completed for untreated control animals. Task i is not complete but stem cells have been successfully harvested and given to recipient animals.

Specific Aim #2 – Analysis microPET images and compare with histology (Months 10-12)
Overview. Compare outcomes of difference RT schedules in the presence and absence of stem cells.

Specific Aim #2 examines excised tumors using histopathology and compares with microPET analysis (Months 10-12)

Subtask1: Surgically excise tumor (regrowth) and normal tissues from treatment animals.

a. Surgically extract intrapulmonary tumors
b. Block and section tissue for histological examination
c. Cut sections and stain with H&E and specific immunochemistry

Subtask2: Compare histology with non-invasive tumor imaging (Months 3-12)

a. Mathematically compare **histology** with functional SUV imaging data

b. Analyze entire data set to determine if PERT is more effective than conventional RT for tumor regrowth and the outcome of stem cell therapy, as confirmed by histology and microPET/CT imaging.

The work from Aim 2 is complete for unirradiated animals to develop the assay. Sub-tasks are not fully complete for animals given standard RT or PERT.